Original Article

Artemether and Lumefantrine Loaded Self-nanoemulsifying drug Delivery System for Enhancement of Bioavailability

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ABSTRACT

Introduction: This study involves development and evaluation of bioavailability of oral self-nanoemulsifying drug delivery system of BCS class II and IV drugs, Artemether and Lumefantrine (AL), respectively. This fixed combination is used for treatment of drug resistant malaria. Self nanoemulsifying drug delivery system (SNEDDS) was developed due to lipophilicity of both drugs. Pseudo ternary phase diagrams were derived based on solubility of drugs in oils and surfactants for identifying self-nanoemulsifying region. **Materials and Methods:** Propylene glycol dicaprylate caprate, Cremophor EL, Tween 80 (1:1) and Transcutol HP were selected as oil and surfactants. Pseudo ternary plots were constructed based on solubility of AL in oils and surfactants to identify composition of formulations. They were evaluated for self-emulsification time, percent transmittance, cloud point, thermodynamic stability and *in vitro* release. Globule size analysis was done using Malvern Zeta sizer. Pharmacokinetic parameters like area under curve (AUC), C_{max} and T_{max} were evaluated using Wistar rats. **Results and Discussion:** All formulations displayed globule size between 27-32 nm while percent transmittance was between 90-99%. Cloud point above 37°C was indicative of integrity of self-nanoemulsifying properties *in vivo*. Cumulative percent release in 1 hr in 0.1 N HCl was in range of 75 to 100 %. A two-fold enhancement in bioavailability was observed with SNEDDS as compared to plain drugs. AUC_{0-5h} were increased by 2 times for artemether and 1.71 times for lumefantrine compared to plain drug suspensions. This proved the prospective use of SNEDDS to improve dissolution and oral bioavailability for poorly water-soluble antimalarial drugs.

Key words: Artemether, Lumefantrine, Low oral bioavailability, Self-nanoemulsifying drug delivery system, *In vitro* dissolution.

INTRODUCTION

Nearly 30% of the world's population is affected by parasitic infections especially third world countries.[1] Amongst various parasitic infections, malaria is the most life-threatening disease. In 2019, more than 229 million people were affected by malaria and 409,000 deaths. An estimated 94% of deaths in 2019 were in the African Region.[2] Existing chemotherapy for malaria includes limited number of clinically effective antimalarial agents. Although treatment for malaria has been successful, the clinical utility of many antimalarial agents is hampered due to poor oral bioavailability and emergence of resistant parasite strains. Paradoxically, since the infection is majorly prevalent in third world countries the economic benefits to pharmaceutical companies is insignificant to drive research for the development of new anti-malarial agents. This scenario has enforced combined use of current antimalarial agents to reduce drug resistance of parasite strain. Adopting smart formulation technologies to maximize or optimize the therapeutic potential of combination drugs is hence the need of the hour.[3]

Combination of Artemether (ART) and Lumefantrine (LUM) was first registered in 1992 in Peoples Republic of China

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and then it was used in 1999 in Europe to treat drug-resistant *Plasmodium falciparum*. It was also included in WHO list of essential medicines.[4,5] ART and LUM work synergistically resulting in rapid clearance of parasitaemia and prevention of recrudescence.[6] The fixed combination of ART and LUM (AL) is used in a 1:6 proportion.[7] ART is a semisynthetic chiral acetal derived from the naturally occurring substance Artemisinin in Figure 1(a). LUM is a racemic mixture of a synthetic Fluorene derivative and it belongs to the aryl-amino-alcohol family in Figure 1(b). However, these drugs are highly lipophilic with ART[a] having CLogP=3.85 and CLogP=10.2 of LUM[a] (a = Calculated using Chemdraw ultraversion) and belong to BCS class II/IV group with low oral bioavailability (ART=40%, LUM=1.62%), which stems from poor solubility of AL in water.[8-11] Extensive literature survey indicates that the absorption of AL can be substantially increased when co-administered with a fatty meal.[12,13] Many studies have shown that various formulation approaches have been tried for β-ART alone to increase its bioavailability.[13,14] However, there was no reported formulation approach attempted to enhance oral absorption of the combination AL although it has marked role in antimalarial therapy.

Lipid formulation systems (LFS) are capable of increasing bioavailability of lipophilic drugs by increase in solubility in gastric fluids, fine droplet size and lymphatic absorption.[15] Self nanoemulsifying drug delivery system (SNEDDS) has gained prominence after successful commercialization of cyclosporine A (Sandimmune® Neoral). Oils, surfactants and co-surfactants in appropriate combination form SNEDDS.[16] Exposure to GI fluids leads to spontaneous oil in water emulsification, with the globule size in the range of 20–200 nm.[17] Since drugs are in molecular state in SNEDDS rate limiting poor solubility of BCS class II and IV drugs is circumvented.

The marketed dosage forms available for AL are tablets (Coartem and Riamet) and dry powder for reconstitution of suspension (Co-Artesiane® suspension). The dose of marketed formulation of ART and LUM combination should be followed by food or drinks rich in fat such as milk.[8] Patients with acute malaria are frequently averse to food and also show uncontrolled fluctuations in AL plasma levels when taken with food.[4] SNEDDS decrease the food effect for drug and reduce fluctuations in the pharmacokinetic properties of drug.[18]

The objective of the present studies was to formulate self nanoemulsifying drug delivery system of combined antimalarial agent (AL) and evaluation of bioavailability.

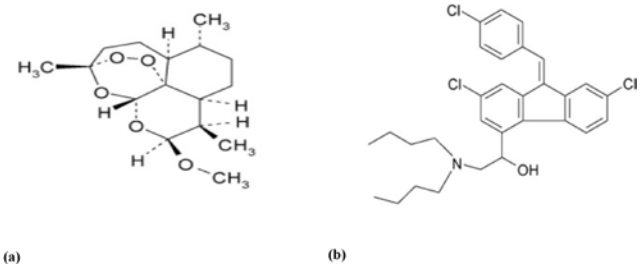

Figure 1: Structure of (a) Artemether (b) Lumefantrine.

MATERIALS AND METHODS

Materials

ART and LUM were gifted by Calyx Chemicals and Pharmaceuticals Ltd. (Mumbai, India). HARIOL® Propylene glycol dicaprylate caprylic acid (PDCC) was gift sample from Subhash Chemical Industries Pvt Ltd. (Pune, India). CREMOPHOR EL (PEG-35-castor oil), CREMOPHOR RH 40 (Polyoxyl 40 hydrogenated castor oil Glycerol polyethylene glycol oxystearate), SOLUTOL HS 15(PEG 660 hydroxy stearate Macrogol 15 hydroxy stearate) were kindly provided by BASF India Ltd (Mumbai, India). TRANSCUTOL®HP (purified diethylene glycol monoethyl ether), LABRASOL® (PEG-8 glycol caprylate), PECEOL (Glyceryl monooleate), LABRAFAC LIPOPHILE, LABRAFILL were kindly obtained by Gattefosse (Mumbai, India). Tween 80 (Polysorbate 80), triacetin and isopropyl myristate was purchased from SD Fine Chemicals. Tri fluoro acetic acid (HPLC grade) was procured from Merck (India). Acetonitrile, Methanol (HPLC grade), acetic acid, formic acid and ammonium acetate (analytical grade) were purchased from Loba Chemicals (Mumbai, India).

Solubility studies

The solubility of AL in different oils and surfactants was determined by shake flask method. PDCC used as oil phase, Cremophor and Tween 80 as surfactants and transcutol as co-surfactant. The surfactants were melted before solubility studies wherever required. Briefly, an excess of AL was added separately to the oils, surfactants and cosurfactant (5 g each) in screw capped vials. Then the mixtures were vortexed for 10 min using a cyclomixer for proper mixing of AL with the vehicles. Mixtures were shaken for 48 hr in a mechanical shaker (Remi, Mumbai, India) maintained at 25 ± 2°C following which centrifugation was done at 5000 rpm for 10 min. The supernatant (0.5 ml) was diluted and supernatant was analyzed for AL by HPLC (Shimadzu, USA).[19]

Pseudo-ternary phase diagrams

Oils and surfactants/cosurfactants (S_{mix}) were selected based on solubility studies.[20] Briefly oil:S_{mix} were taken

in ratio 3:2:1, from which 100 mg mixture was taken and diluted to 100 ml with distilled water and percent transmittance was measured at 547 nm using UV spectrophotometer (Jasco V-530). Phase changes taking place in different S_{mix}-oil compositions in presence of water was studied and phase diagrams were plotted. Different mixtures of surfactants and cosurfactants were prepared in v/v ratios as 1:1, 2:1,3:1 and 4:1. Oil and S_{mix} were mixed uniformly in different volume ratios (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2 and 1:1). Distilled water was added from a burette dropwise to the different mixtures of oil/surfactant/cosurfactant in order to identify the end point which was indicated by formation of a cloudy dispersion. Pseudo-ternary plots were constructed using Chemix school 4.00.

Thermodynamic stability studies

SNEDDS formulations were subjected to thermodynamic stability stress studies with heating and cooling cycles at each low and high temperature (4°C and 45°C) for 48 hr. Formulations were also subjected to six freeze thaw cycles at -20°C and 25°C for 48 hr at each temperature. Subsequently the samples were centrifuged at 3500 rpm for 30 min. Samples were visually observed for phase separation.

Self-emulsification time

Visual evaluation is the primary means of self-emulsification assessment. Each formulation (1 g) was introduced into 500 ml of distilled water in a glass flask at room temperature and stirred gently using magnetic stirrer. The formulations were visually assessed by grading systems given in Table 1.

Evaluation of Drug Loaded SNEDDS

Percentage transmittance

One ml of each formulation was diluted 100-fold and 1000-fold with water, 0.1N HCl and phosphate buffer pH 7.4 separately and percentage transmittance was determined using Jasco UV-Vis spectrophotometer at 547 nm using respective reagent as blank.

Cloud point measurement

The formulations were compared for cloud point value wherein one ml of each formulation was diluted 100 folds with water and subjected to gradual increase in temperature on a water bath till the appearance of a visible cloud.

Viscosity measurement

Viscosity was determined using Brookfield DV II RV cone and plate rheometer (Brook field Engineering Laboratories, Inc, Middleboro, MA) with spindle # LV 63. Speed was kept at 100 rpm. Sample was placed in a beaker and suitable spindle placed in it and viscosity was determined. Final reading was taken after attaining constant reading.

Globule size analysis

The globule size was measured with Malvern zetasizer nano zs (Nano ZS, Malvern Instruments, Worcestershire, UK). Light source used was helium-neon gas laser at intensity of 4 mW. The instrument is based on the principle of dynamic light scattering (DLS).

In vitro dissolution studies

Formulations were filled in size '00' hard hydroxy propyl methyl cellulose (HPMC) capsule and the results were compared with 10 % w/w plain drug suspension AL. *In vitro* release profiles of AL SNEDDS and plain drug suspension of AL were studied using USP XXIII apparatus II (Electrolab India, Mumbai, India) at 37± 0.5°C in 900 ml of 0.1 N HCl. Speed of rotation was 100 rpm. Aliquots of 5 ml were removed at 1, 2, 3, 4, 5 h and replaced with fresh media. Quantification of AL released in the dissolution medium was done by HPLC method (Shimadzu, USA). Mobile phase used was Buffer (pH 3): Acetonitrile (40:60 v/v). Flow rate was 1.5 ml/min.

Bioavailability studies of AL SNEDDS in rats

Bioavailability studies of AL SNEDDS were performed after obtaining consent (AISSMS/IAEC/20-21/01-20/CPCSEA/IAEC/PT-05/05-2K11) from the Institutional

Table 1: Visual Assessment of Efficiency of Self-nanoemulsification.

Dispersability and Appearance	Time of Self-Emulsification (min)	Grade
Formulation spreads rapidly in water forming clear and transparent nanoemulsion	<1	A*
Formulation formed transparent, gel like intermediate structure prior to dispersing completely but could form nanoemulsion	3–5	B*
Formulation droplets spread in water to form turbid emulsion	>5	C#
Formulation exhibits poor emulsification with coalescence of oil droplets	NA	D$

** = nanoemulsion # = Emulsion $ = No emulsion formed*

Animal Ethics Committee (IAEC), AISSMS College of Pharmacy. Albino Wistar rats (250-300 gm) were used as the animal model and were kept under standard laboratory conditions (temperature = 25 ± 2°C and 55 ± 5% RH). Six animals were kept in each polypropylene cage with open access to standard laboratory diet (Lipton feed, Mumbai, India) and water, *ad libitum*. Two formulations viz., plain suspension of AL and AL SNEDDS formulation F5, were given orally to Albino Wistar rats (n=6) at a dose of 48 mg/kg and 8 mg/kg of LUM and ART respectively. AL suspension was prepared by milling AL powder with of 1% (w/v) carboxy methylcellulose (CMC) and diluted to definite volume to yield required quantity. Blood samples (0.2 ml) were withdrawn from the retro plexus orbital vein of rat at 0, 5, 15, 60, 120, 180, 360 and 420 min collected in microcentrifuge tubes containing ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant. The collected blood was centrifuged (Remi R-303) at 8000 rpm for 10 min after mixing with the anticoagulant properly. The plasma was separated and stored at -21°C until analysis was carried out.

Liquid chromatography/mass spectroscopy/mass spectroscopy analysis of AL in rat plasma

Plasma concentration of AL was determined by a liquid chromatography/mass spectroscopy/mass Spectroscopy (LC/MS/MS) method.[21] LC/MS/MS assay was performed on a HPLC system (Shimadzu, USA) which was connected to a mass spectrometry system (Applied Biosystem 3200 Q-TRAP, Lab India, Mumbai). Data analysis was done using software Analyst 1.5.1.

Stability studies

The AL-loaded SNEDDS were stored at 40°C/75% RH (Newtronics chamber) for 03 months and evaluated for percent transmittance, drug content and globule size.

RESULTS AND DISCUSSION

Solubility studies

The solubility of AL in different oils, surfactants and cosurfactants were determined. Identifying the oil having maximum solubilising potential for both drugs was important to achieve optimal drug loading.[22] The solubility of both drugs (AL) was highest in PDCC (Figure 2). PDCC is a medium chain triglyceride (MCT) with HLB of 2. It primarily contains diester of capric acid and caprylic acid. MCTs reportedly enhance the absorption of drugs by modifying the tight junctions of cell membrane and PDCC itself was reported to be permeation enhancer.[23-26] Among surfactants and cosurfactants, the drugs exhibited maximum solubility

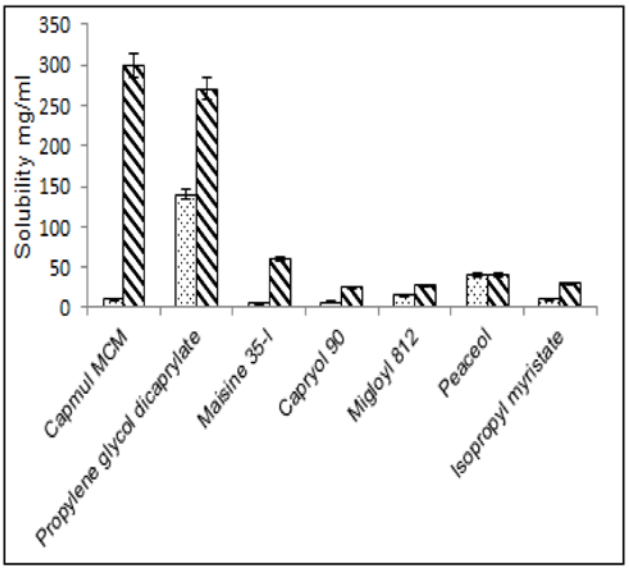

Figure 2: Solubility of Artemether (ART) and Lumefantrine (LUM) in oils.

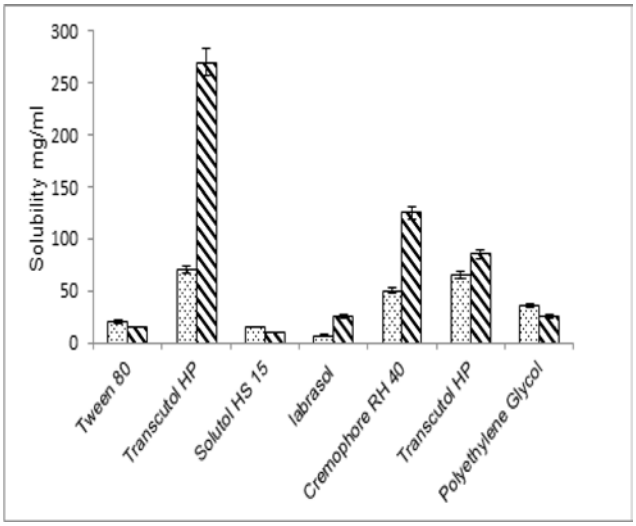

Figure 3: Solubility of ART and LUM in surfactants and cosurfactants. (- SNEDDS ART, -SNEDDS LUM).

in Cremophor ELP and Transcutol HP (Figure 3). However, when emulsification efficiency of Cremophor ELP was evaluated by combining with oil (PDCC) (Table 2) the mixture was found to have poor emulsifying properties. But when combined with tween 80 (in ratio 1:1) the emulsification ability for PDCC was significantly enhanced. This combination was also reportedly used as bioactive enhancer for improvement of dissolution and absorption of hydrophobic drugs such as lacidipine, ramipril and atorvastatin.[27,28] The bioenhancing activity of Cremophor ELP and Tween 80 were attributed to inhibitory effects on p-glycoprotein and Cytochrome P-450 enzymes.[17] The cosurfactant aids to keep the

Table 2: Compositions and physical properties of F3, F4 and F5 formulation.

Composition (% w/w)			
Ingredients	F3	F4	F5
PDCC	34	38	42
Cremophor ELP	22	21.2	20.4
Tween 80	22	21.2	20.4
Transcutol HP	11	10.6	10.2
Artemether	1	1	1
Lumefantrine	6	6	6

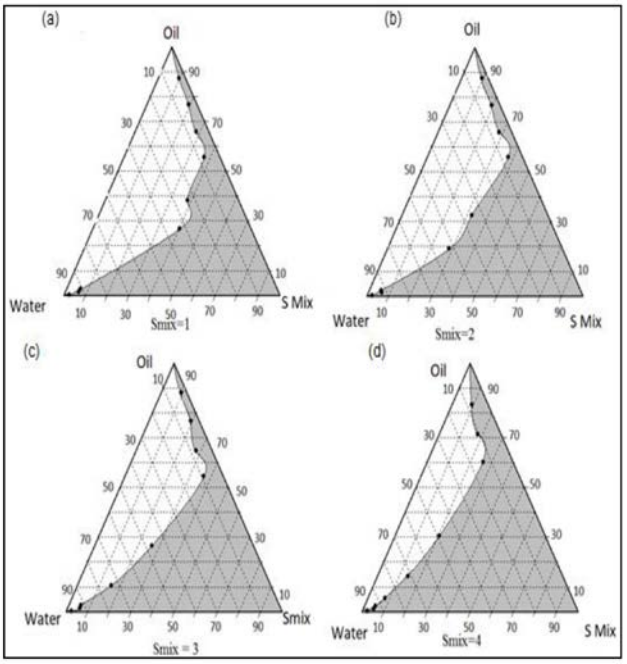

Figure 4: Pseudo ternary phase diagrams. Ratio of surfactant to cosurfactant (v/v) (a) 1:1, (b) 2:1, (c) 3:1 and (d) 4:1.

interfacial barrier film flexible, fluid and tightly packed.[29] Therefore, for present study transcutol HP was selected as a cosurfactant.

Pseudo-ternary phase diagrams

For constructing pseudo-ternary phase diagram, PDCC was selected as oil, while Cremophor and Tween 80 (in 1:1 ratio) were chosen as surfactant and Transcutol HP as a cosurfactant (Figure 4). Nanoemulsion area was found to be greater with an increase in S_{mix} (ratio of concentration of surfactant to cosurfactant) and was highest at S_{mix}= 4:1. At S_{mix}= 4:1 system had capacity to solubilize nearly 50% (w/w) of oily phase (Figure 4 a-d). A bigger nanoemulsion region in the phase diagrams indicates higher nanoemulsifying potential of the combination. Thus one can find regions having better nanoemulsifying ability at lower proportion of surfactant and having higher drug loading potential.[30] Hence further studies were done using 4:1 ratio of S_{mix}. Five formulations were selected containing 30-50% w/w of oil phase (Table 3).

Table 3: Composition of formulations selected from phase diagram.

CODE	Composition (% w/w)	
	Oil	S_{mix}
F1	33	67
F2	37	63
F3	43	57
F4	45	55
F5	50	50

Thermodynamic stability studies

SNEDDS are thermodynamically stable systems which upon contact with GI fluid form micro-emulsion. In nano-emulsions the higher concentration of S_{mix} results in extremely low interfacial tension thereby lowering the entropy of dispersion. This leads to zero or negative free energy which explains the thermodynamic stability of nano-emulsion. Thermodynamic stability studies of formulations were carried out to avoid selection of metastable formulation and to discriminate between nano-emulsion and emulsion. Among all formulations, F1 and F2 showed phase separation during freeze-thaw cycle and centrifugation respectively. The formulations (F3-F5) which passed thermodynamic stability tests were subjected to further tests.

Self-emulsification studies

The rate of self-emulsification is an important parameter affecting the performance of SNEDDS. Series of steps are involved with number of phase changes. The system transforms from swollen w/o reverse micelles systems, to bi-continuous phase and finally to o/w nano-emulsion on dilution. Migration of surfactant from interface can lead to disruption of interfacial barrier film in last transition. This will result in leaching of drug from core of micelle to external environment leading to precipitation.[31] Thus, rate of self- emulsification is critical for any self-emulsifying system. All formulations exhibited self-emulsification within 1 min indicating that they have required *in vivo* stability.

Evaluation of AI-SNEDDS

Percentage transmittance

All formulations (F3-F5) displayed > 90% transmittance after 100 times dilution and 99 % transmittance on 1000

Table 4: Compositions and physical properties of F3, F4 and F5 formulation (*n* = 3, Mean±SD).			
Percentage transmittance (%)			
Contents	F3	F4	F5
Water 100-fold	88.36±0.9131	88±1.00	87±1.00
Water 1000-fold	98.84±0.1652	97.43±1.15	97.63±1.528
0.1 N HCL	99.05±0.2346	98.55±1.15	98.69±0.5774
pH 7.4 buffer 1000-fold	97.49±0.4428	96.12±0.5774	98±1.00
Cloud points	81	79	75
Viscosity	33	30	26
Globule size(nm)	27.53	28.85	32.25
Polydispersity Index	0.568	0.269	0.169

times dilution. As dilution progresses, formulations pass through various phases wherein at low level of dilution most of the oil are in coherent structure as while the higher dilutions, formulations consist of mainly isolated micelles.[32] This is also indicative of nanometer droplet size of the nanoemulsions (Table 4).

Cloud point measurement

Cloud point is a lower consolute temperature characteristically displayed by non-ionic surfactants. Cloud point below 37°C will cause the surfactant to precipitate leading to loss of integrity of the emulsion thereby causing precipitation and leaching of the drug.[33] The nanoemulsion undergoes visible phase changes when the temperature is increased beyond cloud point and reconvert to normal phase on decreasing temperature below cloud point. All formulations showed cloud point above 37°C (Table 4).

Viscosity measurement

Viscosities of all three formulations are mentioned in the Table 4. Formulation F5 showed lesser viscosity (26 cp) as compared to other two formulations. All selected nano-emulsions had very low viscosity. Low viscosity of the formulations is important for large-scale handling as also providing lesser resistance to the diffusion of drug molecules to the external environment. Besides this lower viscosity enables faster self-emulsification (Table 4).

Globule size analysis

Effect of oil phase concentration on the globule size was determined by Malvern Zetasizer. Results of globule size are shown in Table 5. F3 formulation shows minimum globule size 27.53 nm with polydispersibility index 0.584. Although no major difference in globule size (p>0.05) was observed among three formulations, it was found to increase with increase in oil content. Mean globule size of F4 and F3 was 28.85 nm and 27.53 nm respectively which contained 38 % and 34% oil respectively, while mean globule size of formulation F5 was 32.25 nm which contained 42% oil. Globule size in nanometer range implies increased surface area, hence faster release and absorption of drug via lymphatic pathway (Table 4).

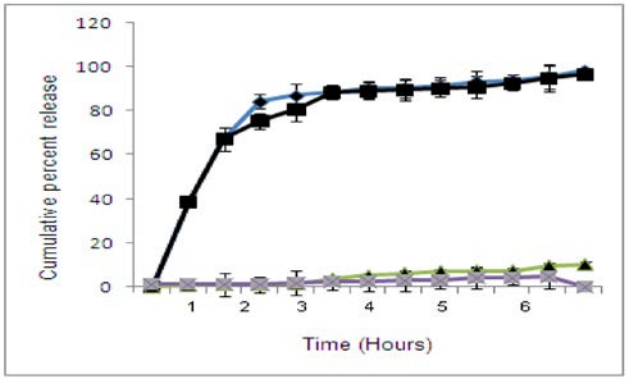

Figure 5: Dissolution profile of (a) F5 SNEDDS (b) F4 SNEDDS (C) F3 SNEDDS in 0.1 N HCL (*n*=3, Mean ±SD).

In vitro drug release

In vitro dissolution profile of formulation F3, F4 and F5 in comparison with simple drug suspension in pH 1.2 is shown in Figure 5. The highest release i.e., 100 % was obtained in case of formulation F5 in 1 h for both drugs. Nearly 89% of ART and 75% LUM released in 15 min as compared to plain drug suspension, which released less than 2% of the both drugs. This difference in release in AL was observed due to difference in log *P* values of both drugs. Log *P* value of ART is 3.83 while that of LUM is 10.2. Hence due to its high lipophilicity, LUM retained for longer time in oil phase and it may restrain the release of the drug into the medium. Interestingly the release from F3 and F4 was significantly (p<0.01) less than F5, although the difference in globule size was not as statistically significant (p >0.05). This may be attributed to the fact

Table 5: Pharmacokinetic parameters of ART when F5 SNEDDS and plain drug suspension were orally administered to male albino wistar rats (*n* = 6, Mean±SD).

Formulation	T_{max} [a] (h)***	C_{max} [b](ng/ml)****	AUC_{0-5h} (ng /ml)	$AUC_{0-\infty}$ (ng/ml)
F5 SNEDDS	1± 0.56	125±13 ng/mL	427.505±56.23	443.505
Plain drug suspension	2±0.4	55.28±8	213.786±23.05	219.046

****p<0.01 and**** p<0.0001 when compared with plain drug suspension using Student's t test*
a=Time of peak concentration.
b=Peak of maximum concentration.

Table 6: Pharmacokinetic parameters of LUM when F5 SNEDDS and plain drug suspension were orally administered to male albino wistar rats (*n* = 6, Mean±SD).

Formulation	T_{max} [a] (h)****	C_{max} [b](ng/ml)****	AUC_{0-5h} [c](ng h/ml)	$AUC_{0-\infty}$
F5 SNEDDS	2±0.82	3100±300	55307.85±760.89	56000.37± 762
Plain drug suspension	6±1.45	1443.35±150	32325.45 ± 456.78	32325.46 ± 457

****p<0.01 and**** p<0.0001 when compared with plain drug suspension using Student's t test*
a=Time of peak concentration.
b=Peak of maximum concentration.

that S_{mix} concentration in F5 is 51 % while in F3 and F4 is 55% and 53% to the oil. The drug in the SNEDDS system exists in molecular state entrapped in the micelles or in the nanoemulsion droplets when diluted into aqueous solution.[34] The polydispersity index was low in case of F5 (Table 4) which also contained 50% of oil to the S_{mix} concentration suggesting formation of more oil globules with uniformity in globule size. Thus, the drug has larger surface area for release and low viscosity in F5 than F3 and F4. In comparison to F5, only 3% LUM and 12 % ART drug released from plain drug suspension in dissolution medium in 1hr. So, release from F5 formulation was significant compared to plain drug suspension.

Bioavailability studies of AL-SNEDDS

Rats were selected for animal studies because linear relationship is reported for absorption in humans and in rats.[35] The dose was calculated based on body surface area formula stated by Shannon Reagan-Shaw *et al.*[36] C_{max} of ART in SNEDDS was enhanced by 2.27 times as compared to the plain ART suspension and marginal increase in T_{max} was seen (Table 5). For LUM SNEDDS the C_{max} was increased by 2 times as compared to the plain LUM suspension. $T_{max.}$ of LUM SNEDDS was increased by 0.33 time (Table 6). The AUC_{0-5h} for plain drug suspension were increased by 2 times for ART and 1.71 times for LUM (Figure 6,7). The mean C_{max} for ART (F5 SNEDDS), 125±13 ng/ml, was reached in 1± 0.56 hr (T_{max}), whereas for LUM (F5 SNEDDS) the C_{max} of 3100±300 ng/ml was reached in 2±0.82 hr. The mean values of AUC_{0-5h} obtained for F5 SNEDDS were 427.505±56.23 and 55307.85±760.89 ng.h/ml for AL,

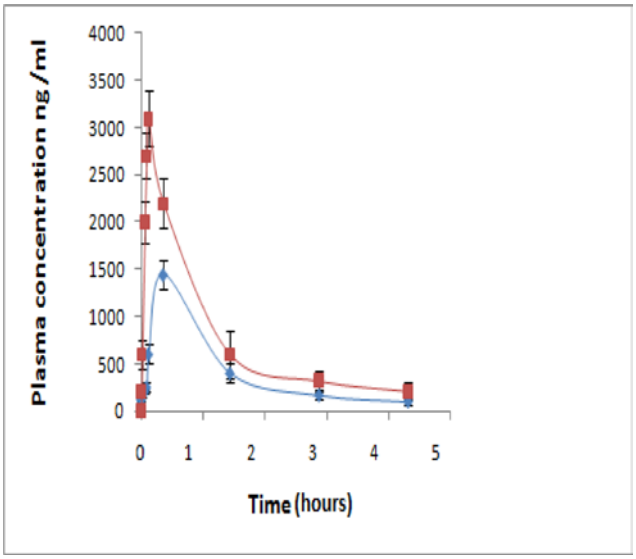

Figure 6: Plasma concentration-time profile of LUM in rat plasma (*n*=3, Mean ±SD).

respectively. Statistically, the difference in T_{max} of ART in F5 SNEDDS was very significant (p<0.01) when compared to T_{max} of plain drug suspension. In case of LUM T_{max} difference between F5 SNEDDS formulation and plain drug suspension were extremely significant (p<0.0001). T_{max} was found to be decreased for both drugs due to presence of drug in nano form and also increased absorption due to solubility enhancement of both drugs. In case of suspension, the drugs are suspended in the form of fine particles and are yet to undergo dissolution in GI fluids to get absorbed. The difference in C_{max} of ART in F5 SNEDDS was extremely significant (p<0.0001) when compared to

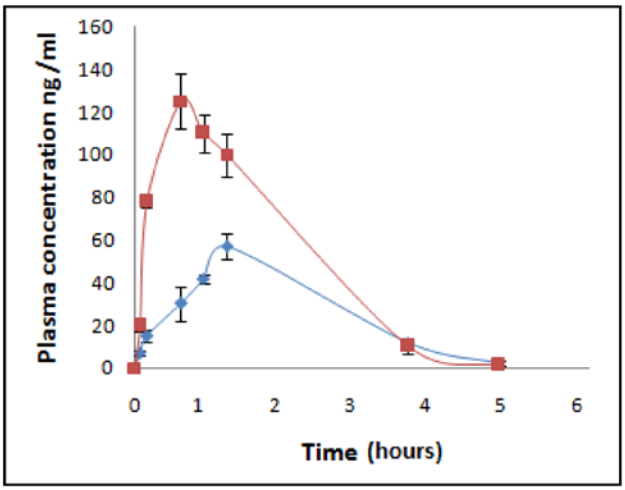

Figure 7: Plasma concentration-time profile of ART in rat plasma (*n*=3, Mean ±SD).

C_{max} of ART in plain drug suspension and in case of LUM the difference in C_{max} between F5 SNEDDS formulation and plain drug suspension was extremely significant ($p<0.0001$). The higher bioavailability can be attributed to presence of Cremophor ELP and Tween 80 which as permeation enhancers. ART and Artemisinin derivatives are known to degrade in acidic environment of gastrointestinal tract.[12] Nanoemulsions have also been reported to prevent drug degradation in the gastrointestinal environment.[28] So, it can be inferred that increased gastric acid stability of ART contributed to bioavailability enhancement of ART.

Stability studies

The F5 formulation subjected to stability studies was evaluated in terms of percent transmission and it was found to be 87% after 3 months. There was no change in drug content in 3 month shows that drugs are chemically stable in SNEDDS. The globule size was found to be 32.25 nm.

CONCLUSION

SNEDDS were successfully formulated to achieve higher release and bioavailability of ART and LUM. Stability was confirmed by thermodynamic stability studies and long-term stability studies. The release and bioavailability from F5 SNEDDS were increased due to presence of drugs in lipidic nano form as well as in dissolved state. Additionally, increased gastric stability of AL contributes increased bioavailability of AL.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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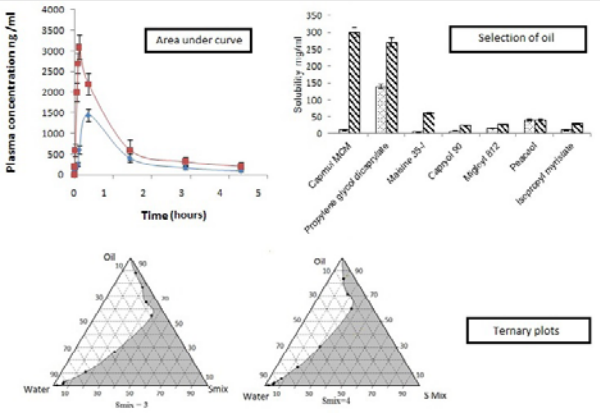
PICTORIAL ABSTRACT



SUMMARY

- Oral self-nanoemulsifying drug delivery system (SNEDDS) of BCS class II drug, Artemether and BCS class IV drug, Lumefantrine, was prepared with the objective of improving bioavailability by resolving their poor aqueous solubility. Both drugs are lipophilic and hence very suitable for SNEDDS. The novelty of the work was using same formulation technology for combination of two drugs. Selection of oils and surfactant-co surfactant (Smix) systems was based on optimal solubility of both drugs. Saturation solubility studies led to selection of PDCC as oil, Cremophor and Tween 80 (in 1:1 ratio) as surfactant and Transcutol HP as a cosurfactant Pseudo ternary phase diagrams facilitated identification of oil-Smix ratio for final formulations. The SNEDDS were found to have globule size below 100 nm. Percent transmittance, cloud point determination and in vitro dissolution studies confirmed that SNEDDS formulations were effective for the two drugs. This was further corroborated by pharmacokinetic studies which showed a significant enhancement for Artemether and Lumefantrine.

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